Networks That Govern Complex Formation during Signal Transduction Exhibit Narrow Flows

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Cell signaling, the biochemical process through which cells sense and respond to their environment, involves a vast and seemingly ever-expanding array of receptors, kinase enzymes, adaptor molecules, and phosphorylation sites, all of which interact in complex ways that vary both subtly and profoundly among different cell types and pathways. Over the past several years it has emerged that early signaling through most receptors in eukaryotic cells involves formation of large, heterogeneous complexes involving these signaling components (reviewed in O'Rourke and Ladbury 2003). The centrality of complex formation is supplanting the previously dominant view that signaling involved a linear or weakly-branched cascade of bimolecular reaction events. Experimental approaches are beginning to map out the temporal evolution of these complexes, although their functional role remains the subject of much speculation. Modeling has a strong role to play in this process since it is nearly impossible to predict the outcome of multi-body interactions without a mathematical model, particularly when those bodies are as complex as a typical signaling protein. Yet surprisingly, nearly all mathematical models of signal transduction to date ignore or greatly simplify complex formation by including only a very small subset of the complexes that can form based on the known interactions of the components.

In this paper we investigate a model for early events in signaling mediated by the high affinity receptor for IgE (FcεRI), a critical component of allergic response, leading to the formation of an activated complex. In a companion paper we explore the role of complex formation by systematically removing assumptions from preexisting models that limit complex formation. The network of complex formation mediated by FcεRI is typical of those that govern complex formation in a wide variety of contexts (Hlavacek et al.) in that the number of basic parameters—total protein concentrations and chemical rate constants—required to define the model is small in comparison with the size of the network. For example, the FcεRI network investigated here is defined by the concentrations of four basic components and 21 rate constants, yet it tracks 354 distinct chemical species, which are coupled through a network of 3680 chemical reactions.

What are the dynamical and functional implications of this very large network of complex formation? We address that question here by analyzing how mass and flux are distributed in the complex formation network and by examining the pathways through the network that transform the basic components into activated complexes. When the component concentrations and rate parameters are varied, mass and flux analysis reveal that under most conditions only a small portion of the signaling network is active, but this active portion can change dramatically with conditions. The comparatively large number of observed pathways—hundreds to thousands—indicates that despite the limited number of active states and reactions, the number of activation pathways remains much larger because of flexibility in the ordering of reaction events leading to activation. *Narrow flows arise here not from the imposition of additional steric constraints that limit what complexes can form, but from the structure and parameters of the network itself, thus indicating its capacity to achieve a high degree of specificity in signaling.*

Model Summary. Detailed descriptions of our model for early events in signaling through FcεRI can be found in previous publications (Faeder et al. 2003; Goldstein et al., 2002) and in interactive form on our website. The model includes four components—a bivalent ligand, the FcεRI receptor, and the kinases Lyn and Syk. The receptor is composed of three distinct subunits, the primarily extracellular α subunit that binds to the ligand, and the primarily cytoplasmic β and γ_2 subunits that contain immunoreceptor tyrosine-based activation motifs (ITAMs), which upon phosphorylation bind to the SH2 domains of Lyn and Syk respectively. Lyn also associates weakly with the unphosphorylated β subunit. The basic events leading to Syk activation in the model can be summarized as follows. Ligand-receptor binding induces dimerization of receptors, which permits Lyn that is weakly associated with receptors to phosphorylate the ITAMs of the *trans* receptor in the dimer, leading to the recruitment of additional Lyn and Syk. Syk in dimers can be transphosphorylated on its

linker region tyrosines by Lyn or on its kinase activation loop tyrosines by Syk.

Analysis of receptor complexes containing active Syk. Of the 164 dimer complexes containing autophosphorylated Syk (Syk*) only 5–10% are typically required to account for 95% of the Syk* at any given time following stimulation with ligand. For our model of the RBL cell line, 12 complexes account for 95% of the Syk* observed following stimulation with bivalent ligand. Despite the narrowness of this distribution, the top two states, which together account for more than 50% of Syk* at steady state, display nearly the full range of variation possible. As shown in Fig. 1, the top complex at steady state

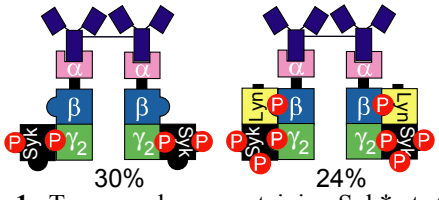

Figure 1. Top complexes containing Syk* at steady state.

contains two Syk*, but no Lyn, and neither Syk* is linker-phosphorylated (an event that requires co-complexation with Lyn). The second most important complex, which exhibits faster kinetics, contains two Lyn and two linker-phosphorylated Syk*. Increasing the total concentration of Lyn narrows the output distribution slightly and increases the total amount of Syk* by nearly ten-fold. At the same time, complexes containing no Lyn diminish in frequency by more than ten-fold. *Thus, changing a single parameter in the model over a range that could occur in the biological system can dramatically affect the distribution of observed complexes.*

Systematic variation of parameters. To examine the effect of parameter variation more systematically, we generated new parameter sets starting from the RBL model by scaling each parameter in the model by a random amount taken from a uniform log-space distribution. Five thousand parameter sets each were generated for up to 2-fold and up to 10-fold scalings. The results from both sets indicate that narrow output state distributions are a robust feature of the model, but they also display a wide range of variation both in the identity of the important complexes and in averaged quantities that could be derived from these, such as the level of linker-phosphorylated Syk*. *In other words, while the output is almost always narrowly distributed, the identity and properties of the important states are sensitive to the parameters of the network.*

Analysis of reaction fluxes. A distinguishing feature of complex formation networks is that large numbers of reactions share the same rate constant. We observe wide variations of flux within these "reaction classes" due to mass variation among the reacting species. As we observed in the distribution of Syk*, a small number of reacting species dominates the reaction flux in all classes. For the RBL model, 10% of the reactions in the network account for 95% of the flux in all classes. Similarly narrow distributions are observed in the randomly-scaled parameter sets.

Analysis of activation pathways. Using either the steady state or time-evolving concentrations of the network components to compute branching probabilities at each step, we compute the probability for an individual molecule to follow a particular sequence of reactions on its way to activation. We call each such sequence a path, and use a stochastic algorithm to sample the huge number of paths that arise in complex formation networks. We have traced activation pathways for both Syk and receptors, and find in both cases that a small number of pathways account for a disproportionate fraction of the flux, but much larger numbers of pathways are required to account for network flow than either species or reactions. Pathway analysis also identifies important intermediate states that may not show up in either mass or flux distributions, and is thus a potentially vital supplement to both.

Outlook. We have described here a new class of biological networks that describes the process of complex formation in signal transduction. The models investigated so far exhibit highly restricted flows through these networks. We plan to conduct more systematic studies of this class of networks to provide more complete explanations of how the vast possibilities afforded by complex formation are transformed into the highly specific outcomes that arise in signaling.

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